

Devi, S.  
09/830026

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Set	Items	Description
S1	604	INVASIN? ? OR INV(5N)INVASIN? ?
S2	16	S1 AND (DENATUR? OR GUANIDINE(W) (HCL OR HYDROCHLORIDE OR H-YDRO(W)CHLORIDE) OR UREA OR DETERGENT? ?)
S3	0	S1 AND DE(W)NATUR?
S4	24	S1(5N) (PURE OR PURIF?)
S5	40	S2 OR S4
S6	29	RD (unique items)

- key terms

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6/3,AB/1 (Item 1 from file: 35)  
DIALOG(R)File 35:Dissertation Abs Online  
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01880296 AADAAI3046704  
Crystallographic studies of \*invasin\*\*\*, a bacterial adhesion molecule from Yersinia pseudotuberculosis  
Author: Hamburger, Zsuzsa Andrea  
Degree: Ph.D.  
Year: 2002  
Corporate Source/Institution: California Institute of Technology (0037)  
Source: VOLUME 63/03-B OF DISSERTATION ABSTRACTS INTERNATIONAL.  
PAGE 1169. 137 PAGES  
ISBN: 0-493-61072-3

Bacterial pathogens, such as *Yersinia pseudotuberculosis*, must bind and enter normally non-phagocytic cells to establish infection. The protein responsible for mediating uptake of the bacterium is a 986-residue outer membrane protein called \*invasin\*\*\*. \*Invasin\*\*\* binds to several members of the  $\beta_1$  integrin family, presumably activating a reorganization of the host cytoskeleton to form pseudopods that envelop the bacterium. Integrin binding has been localized to the extracellular region of \*invasin\*\*\* (Inv497) comprised by

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the COOH-terminal 497 residues. In order to gain insight into host cell entry by *<italic>Yersinia pseudotuberculosis</italic>*, we solved the 2.3 Å crystal structure of Inv497. The structure reveals five domains that form a 180 Å rod with structural similarities to tandem fibronectin-III domains. The integrin-binding surfaces of \*invasin\*\* and fibronectin include similarly located key residues, but in the context of different folds and surface shapes. The structures of \*invasin\*\* and fibronectin provide an example of convergent evolution, in which \*invasin\*\* presents an optimized surface for integrin binding compared with host substrates. We have also initiated structural analyses of the NH<sub>2</sub>-terminal ~500 residues of \*invasin\*\*, which are required for outer membrane localization and for presentation of the integrin-binding region of \*invasin\*\*. We expressed this region of \*invasin\*\* as inclusion bodies in *<italic>E. coli</italic>*, and refolded the protein in the presence of \*detergents\*\*. We have also obtained microcrystals of this membrane protein. Circular dichroism studies indicate that this region of \*invasin\*\* is composed of mainly β-structure. As the transmembrane regions of outer membrane proteins of known structure are β-barrels, this region of \*invasin\*\* is also presumed to fold into such a structure. Proteolysis experiments suggest that the N-terminal 70 amino acids of \*invasin\*\* may form a separate domain from the \*invasin\*\* transmembrane region, analogous to that found in another outer membrane protein, the sucrose-specific porin ScyY. Equilibrium sedimentation analytical ultracentrifugation studies indicate the protein is monomeric in solution. Black bilayer experiments suggest that this region of the protein does not contain a pore and thus plays the role of an outer membrane anchor for the presentation of the integrin-binding domain on the cell surface.

6/3,AB/2 (Item 2 from file: 35)  
DIALOG(R) File 35:Dissertation Abs Online  
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01252670 AAD9234197  
ENTRY OF YERSINIA ENTEROCOLITICA INTO EUKARYOTIC CELLS MEDIATED BY THE INV LOCUS  
Author: YOUNG, VINCENT BENSON  
Degree: PH.D.  
Year: 1992  
Corporate Source/Institution: STANFORD UNIVERSITY (0212)  
Source: VOLUME 53/07-B OF DISSERTATION ABSTRACTS INTERNATIONAL.  
PAGE 3301. 123 PAGES

The inv locus of *Yersinia enterocolitica* is sufficient to convert a noninvasive *E. coli* K-12 strain into a microorganism that is able to penetrate cultured eukaryotic cells. The nucleotide sequence of inv reveals an open reading frame corresponding to an 835 amino acid protein that is homologous to the \*invasin\*\* protein from *Yersinia pseudotuberculosis*. A polyclonal antiserum elicited by a synthetic peptide corresponding to the C-terminal 88 amino acids of this open reading frame detected a unique 100-kd protein in cell lysates of *Y. enterocolitica* strain 8081c and in an *E. coli* strain harboring the cloned inv gene. This protein localized to the outer membranes of both microorganisms and was cleaved by low concentrations of extracellular trypsin. HEp-2 cells were shown to attach to surfaces coated with bacterial outer membranes containing \*invasin\*\* and this attachment was destroyed by treatment of the membranes with trypsin.

The mechanism by which \*invasin\*\* mediates the internalization of bacteria by HEp-2 cells and chicken embryo fibroblasts was investigated.

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\*Invasin"-bearing bacteria initially bound the filopodia and the leading edges of cultured cells. Multiple points of contact between the bacterial surface and the surface of the cell ensued and led to the internalization of the bacterium within an endocytic vacuole; the same multi-step process could be induced by an inert particle coated with \*invasin"-containing membranes. Both adherence and internalization were blocked by an antisera directed against the  $\beta$ 1 integrin cell-adherence molecule. Ultra-structural studies of "detergent"-insoluble cytoskeletons from infected cells and immunofluorescence microscopy of phalloidin-labelled cells showed alterations in the structure of the cytoskeleton during the internalization process including the accumulation of polymerized actin around entering bacteria. Bacterial entry was prevented by cytochalasin D indicating that the internalization process requires actin microfilament function. Possible linkages between  $\beta$ 1 containing integrins and the cytoskeleton were examined during the internalization process through the use of protein-specific antibodies and immunofluorescence microscopy. Like actin, the actin-associated proteins filamin, talin and the  $\beta$ 1 integrin subunit were also found to accumulate around entering bacteria. These findings suggest that the \*invasin"-mediated internalization process is associated with cytoskeletal reorganization.

6/3,AB/3 (Item 1 from file: 144)  
DIALOG(R)File 144:Pascal  
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16103170 PASCAL No.: 03-0261181

Intracellular growth of *Legionella pneumophila* gives rise to a differentiated form dissimilar to stationary-phase forms

GARDUNO Rafael A; GARDUNO Elizabeth; HILTZ Margot; HOFFMAN Paul S  
Department of Microbiology and Immunology, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, B3H-4H7, Canada; Division of Infectious Diseases, Department of Medicine, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, B3H-4H7, Canada

Journal: Infection and immunity, 2002, 70 (11) 6273-6283

Language: English

When *Legionella pneumophila* grows in HeLa cells, it alternates between a replicative form and a morphologically distinct "cyst-like" form termed MIF (mature intracellular form). MIFs are also formed in natural amoebic hosts and to a lesser extent in macrophages, but they do not develop *in vitro*. Since MIFs accumulate at the end of each growth cycle, we investigated the possibility that they are *in vivo* equivalents of stationary-phase (SP) bacteria, which are enriched for virulence traits. By electron microscopy, MIFs appeared as short, stubby rods with an electron-dense, laminar outer membrane layer and a cytoplasm largely occupied by inclusions of poly- $\beta$ -hydroxybutyrate and laminations of internal membranes originating from the cytoplasmic membrane. These features may be responsible for the bright red appearance of MIFs by light microscopy following staining with the phenolic Gimenez stain. In contrast, SP bacteria appeared as dull red rods after Gimenez staining and displayed a typical gram-negative cell wall ultrastructure. Outer membranes from MIFs and SP bacteria were equivalent in terms of the content of the peptidoglycan-bound and disulfide bond cross-linked OmpS porin, although additional proteins, including Hsp60 (which acts as an \*invasin\*\* for HeLa cells), were detected only in preparations from MIFs. Proteomic analysis revealed differences between MIFs and SP forms; in particular, MIFs were enriched for an similar 20-kDa protein, a potential marker of development. Compared with SP bacteria, MIFs were 10-fold more infectious by plaque assay, displayed increased

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resistance to rifampin (3- to 5-fold) and gentamicin (10- to 1,000-fold), resisted "detergent"-mediated lysis, and tolerated high pH. Finally, MIFs had a very low respiration rate, consistent with a decreased metabolic activity. Collectively, these results suggest that intracellular *L. pneumophila* differentiates into a cyst-like, environmentally resilient, highly infectious, post-SP form that is distinct from *in vitro* SP bacteria. Therefore, MIFs may represent the transmissible environmental forms associated with Legionnaires' disease.

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6/3,AB/4 (Item 2 from file: 144)  
DIALOG(R)File 144:Pascal  
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15622046 PASCAL No.: 02-0326303  
Enterotoxigenic *Escherichia coli* TibA glycoprotein adheres to human intestine epithelial cells  
LINDENTHAL Christoph; ELSINGHORST Eric A  
Department of Molecular Biosciences, University of Kansas, Lawrence,  
Kansas 66045-2106, United States  
Journal: *Infection and immunity*, 2001, 69 (1) 52-57  
Language: English

Enterotoxigenic *Escherichia coli* (ETEC) is capable of invading epithelial cell lines derived from the human ileum and colon. Two separate invasion loci (*tia* and *tib*) that direct noninvasive *E. coli* strains to adhere to and invade cultured human intestine epithelial cells have previously been isolated from the classical ETEC strain H10407. The *tib* locus directs the synthesis of TibA, a 104-kDa outer membrane glycoprotein. Synthesis of TibA is directly correlated with the adherence and invasion phenotypes of the *tib* locus, suggesting that this protein is an adhesin and "invasin". Here we report the purification of TibA and characterization of its biological activity. TibA was purified by continuous-elution preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Purified TibA was biotin labeled and then shown to bind to HCT8 human ileocecal epithelial cells in a specific and saturable manner. Unlabeled TibA competed with biotin-labeled TibA, suggesting the presence of a specific TibA receptor in HCT8 cells. These results show that TibA acts as an adhesin. Polyclonal anti-TibA antiserum inhibited invasion of ETEC strain H10407 and of recombinant *E. coli* bearing *tib* locus clones, suggesting that TibA also acts as an invasin. The ability of TibA to direct epithelial cell adhesion suggests a role for this protein in ETEC pathogenesis.

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6/3,AB/5 (Item 3 from file: 144)  
DIALOG(R)File 144:Pascal  
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15618664 PASCAL No.: 02-0322908  
Isolation and characterization of a *Shigella flexneri* invasin complex subunit vaccine  
TURBYFILL K Ross; HARTMAN Antoinette B; OAKS Edwin V  
Department of Enteric Infections, Walter Reed Army Institute of Research,  
Silver Spring, Maryland 20910-7500, United States  
Journal: *Infection and immunity*, 2000, 68 (12) 6624-6632

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Language: English

The invasiveness and virulence of *Shigella* spp. are largely due to the expression of plasmid-encoded virulence factors, among which are the invasion plasmid antigens (Ipa proteins). After infection, the host immune response is directed primarily against lipopolysaccharide (LPS) and the virulence proteins (IpaB, IpaC, and IpaD). Recent observations have indicated that the Ipa proteins (IpaB, IpaC, and possibly IpaD) form a multiprotein complex capable of inducing the phagocytic event which internalizes the bacterium. We have isolated a complex of invasins and LPS from water-extractable antigens of virulent shigellae by ion-exchange chromatography. Western blot analysis of the complex indicates that all of the major virulence antigens of *Shigella*, including IpaB, IpaC, and IpaD, and LPS are components of this macromolecular complex. Mice or guinea pigs immunized intranasally with "purified"\*\* "invasin"\*\* complex (invaplex), without any additional adjuvant, mounted a significant immunoglobulin G (IgG) and IgA antibody response against the *Shigella* virulence antigens and LPS. The virulence-specific response was very similar to that previously noted in primates infected with shigellae. Guinea pigs (keratoconjunctivitis model) or mice (lethal lung model) immunized intranasally on days 0, 14, and 28 and challenged 3 weeks later with virulent shigellae were protected from disease ( $P < 0.01$  for both animal models).

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6/3,AB/6 (Item 4 from file: 144)  
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15616849 PASCAL No.: 02-0321079  
Epithelial cell adherence mediated by the enterotoxigenic *Escherichia coli* Tia protein

MAMMARAPPALLIL Joseph G; ELSINGHORST Eric A  
Department of Molecular Biosciences, University of Kansas, Lawrence,  
Kansas 66045-2106, United States

Journal: Infection and immunity, 2000, 68 (12) 6595-6601

Language: English

In vitro studies have shown that enterotoxigenic *Escherichia coli* (ETEC) strains are capable of invading cultured epithelial cells derived from the human ileum and colon. Two separate invasion loci (tia and tib) have previously been isolated from the classical ETEC strain H10407. The tia locus has been shown to direct the synthesis of Tia, a 25-kDa outer membrane protein. Tia is sufficient to confer the adherence and invasion phenotypes on laboratory stains of *E. coli*, suggesting that this protein is an adhesin and "invasin"\*\*. Here we report the "purification"\*\* of Tia and characterize its biological activity. Tia was purified by electroelution of outer membrane proteins that had been separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Purified Tia was labeled with biotin and then shown to bind to HCT8 human ileocecal epithelial cells in a specific and saturable manner. Polyclonal anti-Tia antiserum blocked this binding. These results show that Tia acts as an adhesin. Polyclonal anti-Tia antiserum also inhibited invasion of recombinant *E. coli* bearing tia clones, indirectly suggesting that Tia may also act as an invasin. We predict Tia to contain eight transmembrane amphipathic beta-sheets with four loops that are exposed on the surface of the bacterial cell. A peptide corresponding to 19 residues in one of the four predicted surface-exposed loops inhibits Tia-mediated epithelial cell invasion. Seeding HCT8 cells on

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wells coated with purified Tia reduced Tia-mediated epithelial cell invasion. Together, these results indicate that Tia is an invasin and adhesin that binds a specific receptor on HCT8 cells.

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6/3,AB/7 (Item 5 from file: 144)  
DIALOG(R)File 144:Pascal  
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14837192 PASCAL No.: 00-0520875  
A multi-domain protein for beta SUB 1 integrin-targeted DNA delivery  
FORTUNATI E; EHLERT E; VAN LOO N-D; WYMAN C; EBLE J A; GROSVELD F;  
SCHOLTE B J  
Department of Cell Biology and Genetics, Erasmus University, Rotterdam,  
Netherlands; Institute for Physiological Chemistry, University of Muenster,  
Germany

Journal: Gene therapy : (Basingstoke), 2000, 7 (17) 1505-1515

Language: English

The development of effective receptor-targeted nonviral vectors for use in vivo is complicated by a number of technical problems. One of these is the low efficiency of the conjugation procedures used to couple protein ligands to the DNA condensing carrier molecules. We have made and characterized a multi-domain protein (SPKR) SUB 4 inv, that is designed to target plasmid DNA to beta SUB 1 integrins in remodeling tissue. It contains a nonspecific DNA-binding domain (SPKR) SUB 4 , a rigid alpha-helical linker, and the C-terminal beta SUB 1 integrin binding domain (aa 793-987) of the Yersinia pseudotuberculosis \*invasin"\*\* protein. (SPKR) SUB 4 \*inv"\*\* could be \*purified"\*\* at high yields using a bacterial expression system. We show that (SPKR) SUB 4 inv binds with high affinity to both plasmid DNA and beta SUB 1 integrins. In a cell attachment assay, the apparent affinity of (SPKR) SUB 4 inv for beta SUB 1 integrins is three orders of magnitude higher than that of the synthetic peptide integrin ligand RGDS. (SPKR) SUB 4 inv-plasmid complexes are not active in an in vitro transfection assay. However, transfection efficiencies of plasmid complexes with a cationic lipid micelle (DOTAP/Tween-20) or a cationic polymer (polyethylenimine), are significantly increased in combination with (SPKR) SUB 4 inv. (SPKR) 4inv-mediated transfection can be inhibited by a soluble form of beta SUB 1 integrin, which is evidence for its receptor specificity. In conclusion, (SPKR) SUB 4 inv allows beta SUB 1 integrin-specific targeting of plasmid-carrier complexes, while avoiding inefficient and cumbersome coupling chemistry. The modular design of the expression vector allows production of similar multi-domain proteins with a different affinity. The further development of such complexes for use in vivo is discussed.

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6/3,AB/8 (Item 6 from file: 144)  
DIALOG(R)File 144:Pascal  
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12483845 PASCAL No.: 96-0147675  
Invasin of Yersinia pseudotuberculosis activates human peripheral B cells  
LUNDGREN E; CARBALLEIRA N; VAZQUEZ R; DUBININA E; BRAENDEN H; PERSSON H;  
WOLF-WATZ H

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Univ. Umea, dep. cell molecular biology, 801 87 Umea, Sweden  
Journal: Infection and immunity, 1996, 64 (3) 829-835  
Language: English

The Yersinia pseudotuberculosis cell surface-located protein invasin was found to promote binding between the pathogen and resting peripheral B cells via beta SUB 1 integrin receptors (CD29). B cells responded by expressing several activation markers and by growing. In contrast, T cells did not react, although these cells express CD29. An isogenic invA mutant failed to activate B cells. The mutation could be complemented by providing the invA SUP + gene in trans. \*Purified\*\*\* \*invasin\*\*\* alone did not activate B cells, although it was able to block the binding of bacteria to the cells.

6/3,AB/9 (Item 7 from file: 144)  
DIALOG(R) File 144:Pascal  
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11233642 PASCAL No.: 94-0051555  
A 76-amino acid disulfide loop in the Yersinia pseudotuberculosis invasin protein is required for integrin receptor recognition  
LEONG J M; MORRISSEY P E; ISBERG R R  
Tufts univ. school medicine, Tufts-New England medical cent. hosp., div. rheumatology immunology, Boston MA 02111, USA  
Journal: The Journal of biological chemistry, 1993, 268 (27) 20524-20532  
Language: English  
The Yersinia pseudotuberculosis invasin protein is a 986-amino acid protein that promotes bacterial penetration into mammalian cells by avidly binding multiple beta SUB 1 -chain integrins. A 192-amino acid carboxyl-terminal domain of invasin was previously shown to be sufficient for binding. Evidence is presented here that a 76-amino acid disulfide loop in the integrin binding domain of invasin is required for invasin-mediated cell binding and entry. Bacterial mutants that were altered at either of 2 cysteine residues in the binding domain of \*invasin\*\*\* were completely defective for entry. \*Purified\*\*\* \*invasin\*\*\* protein derivatives altered at either of these cysteines, in contrast to the wild-type invasin, did not promote either cell binding or penetration

6/3,AB/10 (Item 8 from file: 144)  
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10831196 PASCAL No.: 93-0340552  
Very late antigen 4-dependent adhesion and costimulation of resting human T cells by the bacterial beta 1 integrin ligand invasin  
ENNIS E; ISBERG R R; SHIMIZU Y  
Univ. Michigan medical school, dep. microbiology immunology, Ann Arbor MI 48109, USA  
Journal: (The) Journal of experimental medicine, 1993, 177 (1) 207-212  
Language: English  
Bacteria and viruses often use the normal biological properties of host adhesion molecules to infect relevant host cells. The outer membrane bacterial protein invasin mediates the attachment of Yersinia pseudotuberculosis to human cells. In vitro studies have shown that four members of the very late antigen (VLA) integrin family of adhesion molecules, VLA-3, VLA-4, VLA-5, and VLA-6, can bind to invasin. Since CD4 SUP + T cells express and use these integrins, we have investigated the

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interaction of CD4 SUP + T cells with \*purified\*\*\* \*invasin\*\*\*

6/3,AB/11 (Item 9 from file: 144)  
DIALOG(R) File 144:Pascal  
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09109443 PASCAL No.: 90-0277824  
Multiple beta SUB 1 chain integrins are receptors for \*invasin\*\*\*, a protein that promotes bacterial penetration into mammalian cells  
ISBERG R R; LEONG J M  
Tufts univ., school medicine, dep. molecular biology microbiology, Boston MA 02111, USA

Journal: Cell (Cambridge), 1990, 60 (5) 861-871

Language: English

We show here that multiple members of the integrin superfamily of cell adhesion receptors bind the Y. pseudotuberculosis \*invasin\*\*\* protein prior to bacterial penetration into mammalian cells. Affinity chromatography of crude \*detergent\*\*\* extracts demonstrated that integrins containing the subunit structures alpha SUB 3 beta SUB 1 , alpha SUB 4 beta SUB 1 , alpha SUB 5 beta SUB 1 , and alpha SUB 6 beta SUB 1 bound to immobilized \*invasin\*\*\* . Furthermore, phospholipid vesicles containing isolated integrin proteins were able to attach to \*invasin\*\*\*

6/3,AB/12 (Item 1 from file: 440)  
DIALOG(R) File 440:Current Contents Search(R)  
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13419872 References: 16

TITLE: Cloning, expression, and \*purification\*\*\* of the uropathogenic Escherichia coli \*invasin\*\*\* DraD  
AUTHOR(S): Zalewska B; Piatek R; Cieslinski H; Nowicki B; Kur J (REPRINT)  
AUTHOR(S) E-MAIL: kur@altis.chem.pg.gda.pl  
CORPORATE SOURCE: Gdansk Tech Univ, Dept Microbiol, Ul Narutowicza 11-12/PL-80952 Gdansk//Poland/ (REPRINT); Gdansk Tech Univ, Dept Microbiol, /PL-80952 Gdansk//Poland/; Univ Texas, Dept Obstet & Gynecol, /Galveston//TX/77555

PUBLICATION TYPE: JOURNAL

PUBLICATION: PROTEIN EXPRESSION AND PURIFICATION, 2001, V23, N3 (DEC), P 476-482

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PUBLISHER: ACADEMIC PRESS INC, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495 USA

ISSN: 1046-5928

LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: In this study we presented a very efficient expression system, based on pET30LIC/Ek vector, for producing DraD invasin of the uropathogenic Escherichia coli and a one-step chromatography purification procedure for obtaining pure recombinant protein (DraD-C-His(6)). This protein has a molecular weight of 14,818 and calculated pI of 6.6. It contains a polyhistidine tag at the C-terminus (13 additional amino acids) that allowed single-step isolation by Ni affinity chromatography. Also, we obtained specific antibodies against DraD invasin to develop tools for characterizing the expression and biological function of this protein. The amount and quality of DraD-C-His(6) fusion protein purified from E. coli overexpression system seems to be fully appropriate for crystallographic

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studies (soluble form), and for establishing role of the protein in bacterium (cultured cell line interaction and in the internalization process) and for obtaining rabbit polyclonal antisera (insoluble form). (C) 2001 Academic Press.

6/3,AB/13 (Item 2 from file: 440)  
DIALOG(R) File 440:Current Contents Search(R)  
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12903289 References: 23

TITLE: Expression, refolding and crystallization of the OpcA \*invasin\*\*\*  
from *Neisseria meningitidis*

AUTHOR(S): Prince SM; Feron C; Janssens D; Lobet Y; Achtman M; Kusecek B;  
Bullough PA; Derrick JP (REPRINT)

AUTHOR(S) E-MAIL: jeremy.derrick@umist.ac.uk

CORPORATE SOURCE: UMIST, Dept Biomol Sci, POB 88/Manchester M60

1QD/Lancs/England/ (REPRINT); UMIST, Dept Biomol Sci, /Manchester M60

1QD/Lancs/England/; GlaxoSmithKline Biol, /Rixensart//Belgium/; Max  
Planck Inst Infekt Biol, /D-10117 Berlin//Germany/; Univ Sheffield,  
Western Bank, /Sheffield S10 2TN/S Yorkshire/England/

PUBLICATION TYPE: JOURNAL

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2001, V57, , 8 (AUG), P1164-1166

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COPENHAGEN, DENMARK

ISSN: 0907-4449

LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: OpcA is an integral outer membrane from the Gram-negative pathogen *Neisseria meningitidis* that plays a role in adhesion of meningococci to host cells. The protein was overexpressed in *Escherichia coli* in an insoluble form and a procedure developed for refolding by rapid dilution from \*denaturant\*\*\* into \*detergent\*\*\* solution. The refolded material was identical to native OpcA isolated from meningococci, as judged by overall molecular weight, migration on SDS-PAGE and reaction against monoclonal antibodies. Both native and recombinant OpcA crystallized under similar conditions to give an orthorhombic crystal form ( $P2(1)2(1)2$ ), with unit-cell parameters  $a = 96.9$ ,  $b = 46.3$ ,  $c = 74.0$  Angstrom. Complete data sets of reflections were collected from native and refolded OpcA to 2.0 Angstrom resolution.

6/3,AB/14 (Item 3 from file: 440)  
DIALOG(R) File 440:Current Contents Search(R)  
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12147335 References: 28

TITLE: The superantigenic toxin of *Yersinia pseudotuberculosis*: a novel virulence factor?

AUTHOR(S): Carnoy C (REPRINT); Muller-Alouf H; Desreumaux P; Mullet C;  
Granette C; Simonet M

AUTHOR(S) E-MAIL: christophe.carnoy@ibl.fr

CORPORATE SOURCE: Inst Biol Lille, INSERM EPI 9919, 1 Rue Pr  
Calmette/F-59021 Lille//France/ (REPRINT); Univ Lille JE 2225, Inst Biol,  
/F-59021 Lille//France/; Inst Pasteur, Dept Microbiol Ecosyst, /F-59019  
Lille//France/; Ctr Hosp Reg & Univ Lille, Serv Malad Appareil Digestif,

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/F-59037 Lille//France/

PUBLICATION TYPE: JOURNAL

PUBLICATION: INTERNATIONAL JOURNAL OF MEDICAL MICROBIOLOGY, 2000, V290,  
N4-5 (OCT), P477-482

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D-07705 JENA, GERMANY

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LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: Recently, a superantigenic toxin designated YPM (*Yersinia pseudotuberculosis*-derived mitogen) was characterized in the supernatant of *Y. pseudotuberculosis* a Gram-negative bacterium involved in human enteric infection. To assess the role of YPM in pathophysiology of *Y. pseudotuberculosis*, a superantigen-deficient mutant was constructed and its virulence was tested in a murine model of infection and compared with the virulence of the wild-type strain (wt). Determination of the survival rate after intravenous inoculation of mice clearly demonstrated a higher survival rate when animals were infected with the superantigen-deficient strain. This decreased virulence of the mutant strain could not be explained by a lower bacterial growth rate in spleen, liver or lung of infected animals. Therefore, production of IFN gamma, TNF alpha, IL-2, IL-6 and IL-10 was followed during the course of infection by cytokine assay in the blood and mRNA detection in the spleen. IL-6 and IFN gamma were the two major cytokines detected whereas TNF alpha production was never observed.

6/3,AB/15 (Item 4 from file: 440)  
DIALOG(R)File 440:Current Contents Search(R)  
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11962488 References: 40

TITLE: A multi-domain protein for beta(1) integrin-targeted DNA delivery

AUTHOR(S): Fortunati E; Ehrlert E; van Loo ND; Wyman C; Eble JA; Grosveld F;  
Scholte BJ (REPRINT)

CORPORATE SOURCE: Erasmus Univ, Dept Cell Biol & Genet, POB 1738/NL-3000 DR  
Rotterdam//Netherlands/ (REPRINT); Erasmus Univ, Dept Cell Biol & Genet,  
/NL-3000 DR Rotterdam//Netherlands/; Univ Munster, Inst Physiol Chem,  
/D-4400 Munster//Germany/

PUBLICATION TYPE: JOURNAL

PUBLICATION: GENE THERAPY, 2000, V7, N17 (SEP), P1505-1515

GENUINE ARTICLE#: 351QD

PUBLISHER: NATURE PUBLISHING GROUP, HOUNDMILLS, BASINGSTOKE RG21 6XS,  
HAMPSHIRE, ENGLAND

ISSN: 0969-7128

LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: The development of effective receptor-targeted nonviral vectors for use in vivo is complicated by a number of technical problems. One of these is the low efficiency of the conjugation procedures used to couple protein ligands to the DNA condensing carrier molecules. We have made and characterized a multi-domain protein (SPKR)(4)inv, that is designed to target plasmid DNA to beta(1) integrins in remodeling tissue. It contains a nonspecific DNA-binding domain (SPKR)(4), a rigid or-helical linker, and the C-terminal beta(1) integrin binding domain (aa 793-987) of the *Yersinia pseudotuberculosis* \*invasin\*\* protein. (SPKR)(4)\*inv\*\* could be \*purified\*\* at high yields using a bacterial expression system. We show that (SPKR),inv binds with high affinity to both plasmid DNA and beta(1)

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integrins. in a cell attachment assay, the apparent affinity of (SPKR)(4)inv for beta(1) integrins is three orders of magnitude higher than that of the synthetic peptide integrin ligand RGDS. (SPKR)(4)inv-plasmid complexes are not active in an in vitro transfection assay. However, transfection efficiencies of plasmid complexes with a cationic lipid micelle (DOTAP/Tween-20) or a cationic polymer (polyethylenimine), are significantly increased in combination with (SPKR),inv. (SPKR),inv-mediated transfection can be inhibited by a soluble form of beta(1) integrin, which is evidence for its receptor specificity. In conclusion, (SPKR),inv allows PI integrin-specific targeting of plasmid-carrier complexes, while avoiding inefficient and cumbersome coupling chemistry. The modular design of the expression vector allows production of similar multi-domain proteins with a different affinity. The further development of such complexes for use in vivo is discussed.

6/3,AB/16 (Item 5 from file: 440)  
DIALOG(R)File 440:Current Contents Search(R)  
(c) 2003 Inst for Sci Info. All rts. reserv.

11651427 References: 21  
TITLE: Interaction of *Shigella flexneri* IpaC with model membranes correlates with effects on cultured cells  
AUTHOR(S): Tran N; Serfis AB; Osiecki JC; Picking WL; Coye L; Davis R; Picking WD (REPRINT)  
AUTHOR(S) E-MAIL: picking@eagle.cc.ukans.edu  
CORPORATE SOURCE: Univ Kansas, Dept Mol Biosci, 8047 Haworth/Lawrence//KS/66045 (REPRINT); Univ Kansas, Dept Mol Biosci, /Lawrence//KS/66045; St Louis Univ, Dept Chem, /St Louis//MO/63103; St Louis Univ, Dept Biol, /St Louis//MO/63103  
PUBLICATION TYPE: JOURNAL  
PUBLICATION: INFECTION AND IMMUNITY, 2000, V68, N6 (JUN), P3710-3715  
GENUINE ARTICLE#: 316LF  
PUBLISHER: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA  
ISSN: 0019-9567  
LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: Invasion of enterocytes by *Shigella flexneri* requires the properly timed release of IpaB and IpaC at the host-pathogen interface; however, only IpaC has been found to possess quantifiable activities in vitro. We demonstrate here that when added to cultured cells, purified IpaC elicits cytoskeletal changes similar to those that occur during *Shigella* invasion. This IpaC effect may correlate with its ability to interact with model membranes at physiological pH and to promote entry by an ipaC mutant of *S. flexneri*.

6/3,AB/17 (Item 6 from file: 440)  
DIALOG(R)File 440:Current Contents Search(R)  
(c) 2003 Inst for Sci Info. All rts. reserv.

10378272 References: 48  
TITLE: A region of the *Yersinia pseudotuberculosis* invasin protein enhances integrin-mediated uptake into mammalian cells and promotes self-association  
AUTHOR(S): Dersch P; Isberg RR (REPRINT)  
AUTHOR(S) E-MAIL: risberg@opal.tufts.edu

09/830026

CORPORATE SOURCE: Tufts Univ, Dept Mol Biol & Microbiol, 136 Harrison Ave/Boston//MA/02111 (REPRINT); Tufts Univ, Dept Mol Biol & Microbiol, /Boston//MA/02111; Howard Hughes Med Inst, /Boston//MA/02111

PUBLICATION TYPE: JOURNAL

PUBLICATION: EMBO JOURNAL, 1999, V18, N5 (MAR 1), P1199-1213

GENUINE ARTICLE#: 177AH

PUBLISHER: OXFORD UNIV PRESS, GREAT CLARENDON ST, OXFORD OX2 6DP, ENGLAND

ISSN: 0261-4189

LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: Invasin allows efficient entry into mammalian cells by Yersinia pseudotuberculosis. It has been shown that the C-terminal 192 amino acids of invasin are essential for binding of beta(1) integrin receptors and subsequent uptake. By analyzing the internalization of latex beads coated with invasin derivatives, an additional domain of invasin was shown to be required for efficient bacterial internalization. A monomeric derivative encompassing the C-terminal 197 amino acids was inefficient at promoting entry of latex beads, whereas dimerization of this derivative by antibody significantly increased uptake. By using the DNA-binding domain of lambda repressor as a reporter for invasin self-interaction, we have demonstrated that a region of the invasin protein located N-terminal to the cell adhesion domain of invasin is able to self-associate. Chemical crosslinking studies of \*purified\*\* and surface-exposed \*invasin\*\* proteins, and the dominant-interfering effect of a nonfunctional invasin derivative are consistent with the presence of a self-association domain that is located within the region of invasin that enhances bacterial uptake. We conclude that interaction of homomultimeric invasin with multiple integrins establishes tight adherence and receptor clustering, thus providing a signal for internalization.

6/3,AB/18 (Item 7 from file: 440)  
DIALOG(R)File 440:Current Contents Search(R)  
(c) 2003 Inst for Sci Info. All rts. reserv.

07734976 References: 62

TITLE: A region of the Yersinia pseudotuberculosis invasin protein that contributes to high affinity binding to integrin receptors

AUTHOR(S): Saltman LH (REPRINT) ; Lu Y; Zaharias EM; Isberg RR

CORPORATE SOURCE: TUFTS UNIV,SCH MED, HOWARD HUGHES MED

INST/BOSTON//MA/02111 (REPRINT); TUFTS UNIV,SCH MED, HOWARD HUGHES MED

INST/BOSTON//MA/02111; TUFTS UNIV,SCH MED, DEPT MOL BIOL &

MICROBIOL/BOSTON//MA/02111

PUBLICATION TYPE: JOURNAL

PUBLICATION: JOURNAL OF BIOLOGICAL CHEMISTRY, 1996, V271, N38 (SEP 20), P 23438-23444

GENUINE ARTICLE#: VH768

PUBLISHER: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814

ISSN: 0021-9258

LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: The entry of Yersinia pseudotuberculosis into cultured mammalian cells is mediated by the bacterial protein invasin. The mammalian receptors for invasin are five beta(1) chain integrins. Site directed mutagenesis of the aspartate and lysine residues in the 192-amino acid integrin binding domain of invasin was performed to identify regions, in addition to the previously characterized 903-913 region, that are important for integrin

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binding. One mutation, D811A, resulted in depressed ability of \*invasin\*\* to bind \*purified\*\* alpha(5) beta(1) and to promote bacterial entry. Further mutational analysis of Asp-811 indicated that an oxygen-containing side chain is required at this position. A second nearby residue, Phe-808, was also shown to be important for integrin binding, as an alanine substitution at this site had properties similar to the Asp-811 mutation. This mutational analysis has therefore identified a second region that, in conjunction with residues 903-913, is required for wild type levels of integrin binding. The contribution to binding by two noncontiguous sites in the primary sequence parallels results that indicate two domains of fibronectin are involved in integrin binding.

6/3,AB/19 (Item 8 from file: 440)  
DIALOG(R)File 440:Current Contents Search(R)  
(c) 2003 Inst for Sci Info. All rts. reserv.

06133082 References: 58  
TITLE: AN ASPARTATE RESIDUE OF THE YERSINIA PSEUDOTUBERCULOSIS INVASIN PROTEIN THAT IS CRITICAL FOR INTEGRIN BINDING  
AUTHOR(S): LEONG JM; MORRISSEY PE; MARRA A; ISBERG RR (Reprint)  
CORPORATE SOURCE: TUFTS UNIV,SCH MED, HOWARD HUGHES MED  
INST/BOSTON//MA/02111 (Reprint); TUFTS UNIV,SCH MED, HOWARD HUGHES MED  
INST/BOSTON//MA/02111; TUFTS UNIV, NEW ENGLAND MED CTR, DEPT MED, DIV  
RHEUMATOL/BOSTON//MA/02111; TUFTS UNIV, SCH MED, DEPT MOLEC BIOL &  
MICROBIOL/BOSTON//MA/02111  
PUBLICATION: EMBO JOURNAL, 1995, V14, N3 (FEB 1), P422-431  
GENUINE ARTICLE#: QF718  
ISSN: 0261-4189  
LANGUAGE: ENGLISH DOCUMENT TYPE: ARTICLE

ABSTRACT: The Yersinia pseudotuberculosis invasin protein mediates bacterial entry into mammalian cells by binding multiple beta(1)-chain integrins. \*Invasin\*\* binding to \*purified\*\* alpha(5) beta(1) integrin is inhibited by Arg-Gly-Asp (RGD)-containing peptides, although invasin contains no RGD sequence. Fifteen mutations that diminished binding and bacterial entry were isolated after mutagenesis of the entire inv gene. All of the mutations altered residues within the C-terminal 192 amino acids of invasin, previously delineated as the integrin binding domain, and 10 of the mutations fell within an 11 residue region. This small region was subjected to site-directed mutagenesis and almost half of the 35 mutations generated decreased invasin-mediated entry. D911 within this region was the most critical residue, as even a conservative glutamate substitution abolished bacterial penetration. \*Purified\*\* \*invasin\*\* derivatives altered at this residue were defective in promoting cell attachment and this defect was reflected in a 10-fold or greater increase in IC50 for integrin binding. D911 may have a function similar to that of the aspartate residue in RGD-containing sequences.

6/3,AB/20 (Item 9 from file: 440)  
DIALOG(R)File 440:Current Contents Search(R)  
(c) 2003 Inst for Sci Info. All rts. reserv.

04703080 References: 29  
TITLE: IMMUNOLOGIC RECOGNITION OF A 25-AMINOACID REPEAT ARRAYED IN TANDEM ON A MAJOR ANTIGEN OF BLASTOMYCOSIS-DERMATITIDIS  
AUTHOR(S): KLEIN BS; HOGAN LH; JONES JM

09/830026

CORPORATE SOURCE: UNIV WISCONSIN, HOSP & CLIN, 600 HIGHLAND  
AVE/MADISON//WI/53792 (Reprint); UNIV WISCONSIN, SCH MED, DEPT  
PEDIAT/MADISON//WI/53706; UNIV WISCONSIN, SCH MED, DEPT MED MICROBIOL &  
IMMUNOL/MADISON//WI/53706; WILLIAM S MIDDLETON MEM VET ADM MED CTR, RES  
SERV/MADISON//WI/53705

PUBLICATION: JOURNAL OF CLINICAL INVESTIGATION, 1993, V92, N1 (JUL), P  
330-337

GENUINE ARTICLE#: LL773

ISSN: 0021-9738

LANGUAGE: ENGLISH DOCUMENT TYPE: ARTICLE

ABSTRACT: A 120-kD glycoprotein antigen abundantly expressed on Blastomyces dermatitidis yeasts is a target of cellular and humoral immune responses in human infection. To investigate the antigen and immune response more carefully at the molecular level, we screened an expression library from *B. dermatitidis* to identify clones that encode this antigen, designated WI-1. A 942-bp cDNA was isolated by immunologic screening with polyclonal, rabbit anti-WI-1 antiserum. Northern hybridization analysis showed that the cDNA hybridized to yeast message congruent-to 3.9 kb. DNA and deduced protein sequence analysis of the clone demonstrated a 25-amino acid repeat arrayed in tandem, present in 4.5 copies near the 5' end, and rich in predicted antigenic epitopes. Further analysis showed strong homology in these tandem repeats with invasin, an adhesin of *Yersinia*. Cloned cDNA was used to express a 30-kD fusion protein strongly recognized in western blots by rabbit anti-WI-1 antiserum, and by sera from all 35 blastomycosis patients studied. The fusion protein product of subcloned cDNA encoding only the tandem repeat also was strongly recognized in western blots by sera from the 35 blastomycosis patients, but not by sera from 10 histoplasmosis and 5 coccidioidomycosis patients. An antigen-inhibition radioimmunoassay showed that the tandem repeat alone completely eliminated rabbit and human anti-WI-1 antibody binding to radiolabeled native WI-1. From these results, we conclude that the 25-amino acid repeat of WI-1 displays an immunodominant B cell epitope, and that the carboxyl-terminus of the molecule exhibits an architecture that may promote adhesion of *Blastomyces* yeasts to host cells or extracellular matrix proteins and ultimately provide a clearer picture of the molecular pathogenesis of blastomycosis.

6/3, AB/21 (Item 10 from file: 440)  
DIALOG(R) File 440: Current Contents Search(R)  
(c) 2003 Inst for Sci Info. All rts. reserv.

04222783 References: 28

TITLE: VERY LATE ANTIGEN 4-DEPENDENT ADHESION AND COSTIMULATION OF RESTING HUMAN T-CELLS BY THE BACTERIAL BETA-1 INTEGRIN LIGAND INVASION

AUTHOR(S): ENNIS E; ISBERG RR; SHIMIZU Y (Reprint)

CORPORATE SOURCE: UNIV MICHIGAN, SCH MED, DEPT MICROBIOL & IMMUNOL/ANN ARBOR//MI/48109 (Reprint); UNIV MICHIGAN, SCH MED, DEPT MICROBIOL & IMMUNOL/ANN ARBOR//MI/48109; TUFTS UNIV, SCH MED, DEPT MOLEC BIOL & MICROBIOL/BOSTON//MA/02111

PUBLICATION: JOURNAL OF EXPERIMENTAL MEDICINE, 1993, V177, N1 (JAN 1), P  
207-212

GENUINE ARTICLE#: KE652

ISSN: 0022-1007

LANGUAGE: ENGLISH DOCUMENT TYPE: NOTE

ABSTRACT: Bacteria and viruses often use the normal biological properties of host adhesion molecules to infect relevant host cells. The outer

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membrane bacterial protein invasin mediates the attachment of *Yersinia pseudotuberculosis* to human cells. In vitro studies have shown that four members of the very late antigen (VLA) integrin family of adhesion molecules, VLA-3, VLA-4, VLA-5, and VLA-6, can bind to invasin. Since CD4+ T cells express and use these integrins, we have investigated the interaction of CD4+ T cells with \*purified\*\*\* \*invasin\*\*\*. Although VLA integrin-mediated adhesion of T cells to other ligands such as fibronectin does not occur at high levels unless the T cells are activated, resting T cells bind strongly to \*purified\*\*\* \*invasin\*\*\*. The binding of resting T cells to invasin requires metabolic activity and an intact cytoskeleton. Although CD4+ T cells express VLA-3, VLA-4, VLA-5, and VLA-6, monoclonal antibody (mAb) blocking studies implicate only VLA-4 as a T cell invasin receptor. Like other integrin ligands, invasin can facilitate T cell proliferative responses induced by a CD3-specific mAb. These results suggest that the nature of the integrin ligand is a critical additional factor that regulates T cell integrin activity, and that direct interactions of T cells with bacterial pathogens such as *Yersinia* may be relevant to host immune responses to bacterial infection.

6/3,AB/22 (Item 11 from file: 440)  
DIALOG(R) File 440:Current Contents Search(R)  
(c) 2003 Inst for Sci Info. All rts. reserv.

03375819 References: 38

TITLE: THE \*INVASIN\*\*\* PROTEIN OF *YERSINIA-ENTEROCOLITICA* - INTERNALIZATION OF \*INVASIN\*\*\*-BEARING BACTERIA BY EUKARYOTIC CELLS IS ASSOCIATED WITH REORGANIZATION OF THE CYTOSKELETON  
AUTHOR(S): YOUNG VB; FALKOW S; SCHOOLNIK GK  
CORPORATE SOURCE: STANFORD UNIV,DEPT MICROBIOL & IMMUNOL/STANFORD//CA/94305  
(Reprint); STANFORD UNIV,DEPT MED, DIV GEOG MED/STANFORD//CA/94305;  
STANFORD UNIV, HOWARD HUGHES MED INST/STANFORD//CA/94305  
PUBLICATION: JOURNAL OF CELL BIOLOGY, 1992, V116, N1 (JAN), P197-207  
GENUINE ARTICLE#: GY959  
LANGUAGE: ENGLISH DOCUMENT TYPE: ARTICLE

ABSTRACT: *Yersinia enterocolitica*, a facultative intracellular pathogen of mammals, readily enters (i.e., invades) cultured eukaryotic cells, a process that can be conferred by the cloned *inv* locus of the species. We have studied the mechanism by which the product of *inv*, a microbial outer membrane protein termed "\*invasin\*\*\*," mediates the internalization of bacteria by HEp-2 cells and chicken embryo fibroblasts. \*Invasin\*\*\*-bearing bacteria initially bound the filopodia and the leading edges of cultured cells. Multiple points of contact between the bacterial surface and the surface of the cell ensued and led to the internalization of the bacterium within an endocytic vacuole; the same multistep process could be induced by an inert particle coated with \*invasin\*\*\*-containing membranes. Both adherence and internalization were blocked by an antisera directed against the beta-1 integrin cell-adherence molecule. Ultrastructural studies of \*detergent\*\*\*-insoluble cytoskeletons from infected cells and immunofluorescence microscopy of phalloidin-labeled cells showed alterations in the structure of the cytoskeleton during the internalization process including the accumulation of polymerized actin around entering bacteria. Bacterial entry was prevented by cytochalasin D indicating that the internalization process requires actin microfilament function. Possible linkages between beta-1 containing integrins and the cytoskeleton were examined during the internalization process through the use of protein-specific antibodies and immunofluorescence microscopy. Like actin,

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the actin-associated proteins filamin, talin and the beta-1 integrin subunit were also found to accumulate around entering bacteria. These findings suggest that the "invasin"-mediated internalization process is associated with cytoskeletal reorganization.

6/3,AB/23 (Item 1 from file: 348)  
DIALOG(R)File 348:EUROPEAN PATENTS  
(c) 2003 European Patent Office. All rts. reserv.

01578461

Oligopeptides for promoting hair growth  
Oligopeptide zur Forderung des Haarwuchses  
Oligopeptides pour stimuler la croissance des cheveux  
PATENT ASSIGNEE:

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PATENT (CC, No, Kind, Date): EP 1310511 A2 030514 (Basic)

APPLICATION (CC, No, Date): EP 2002025060 021112;

PRIORITY (CC, No, Date): JP 2001347338 011113; JP 2001371366 011205; JP  
2001371175 011205; JP 2001347340 011113

DESIGNATED STATES: AT; BE; BG; CH; CY; CZ; DE; DK; EE; ES; FI; FR; GB; GR;  
IE; IT; LI; LU; MC; NL; PT; SE; SK; TR

EXTENDED DESIGNATED STATES: AL; LT; LV; MK; RO; SI

INTERNATIONAL PATENT CLASS: C07K-007/04; C07K-007/06; C07K-016/18;  
A61P-017/14

#### ABSTRACT EP 1310511 A2

The present invention provides oligopeptides having morphogenesis promoting activity and in particular, hair promoting activity. The oligopeptides may be in monomer form, monomer having a reactive substance bound form or as a polymer, such as a dimer including a homodimer; heterodimer; homotrimer; or heterotrimer. The present invention also provides monoclonal antibodies that specifically recognize a 220kDa antigen of epithelial new hair follicles; hybridomas producing such antibody; and methods and kits for assaying hair growth in mammalian subjects.

ABSTRACT WORD COUNT: 78

NOTE:

Figure number on first page: 1

09/830026

LANGUAGE (Publication, Procedural, Application): English; English; English  
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	200320	3691
SPEC A	(English)	200320	26497
Total word count - document A			30188
Total word count - document B			0
Total word count - documents A + B			30188

6/3,AB/24 (Item 2 from file: 348)  
DIALOG(R) File 348:EUROPEAN PATENTS  
(c) 2003 European Patent Office. All rts. reserv.

01426571  
Carboxylic acid derivatives that inhibit the binding of integrins to their receptors  
Carbonsaurederivate, die die Bindung von Integrinen an ihre Rezeptoren hemmen  
Derives d'acides carboxyliques inhibant la liaison des intégrines à leurs récepteurs

PATENT ASSIGNEE:

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PATENT (CC, No, Kind, Date): EP 1203766 A2 020508 (Basic)

APPLICATION (CC, No, Date): EP 2001125494 011106;

PRIORITY (CC, No, Date): US 707068 001106; US 973142 011009

DESIGNATED STATES: AT; BE; CH; CY; DE; DK; ES; FI; FR; GB; GR; IE; IT; LI; LU; MC; NL; PT; SE; TR

EXTENDED DESIGNATED STATES: AL; LT; LV; MK; RO; SI

INTERNATIONAL PATENT CLASS: C07D-239/47; C07D-213/75; C07D-237/22;

C07D-215/38; C07D-498/04; A61K-031/505; A61K-031/44; A61K-031/50;

A61K-031/4704; A61P-029/00; A61P-035/00; C07D-498/04; C07D-263:00;

C07D-221:00

ABSTRACT EP 1203766 A2

A method for the inhibition of the binding of ( $\alpha$ 4)( $\beta$ 1)) integrin to its receptors, for example VCAM-1 (vascular cell adhesion molecule-1) and fibronectin; compounds that inhibit this binding; pharmaceutically active compositions comprising such compounds; and to the use of such compounds either a above, or in formulations for the control or prevention of diseases states in which ( $\alpha$ 4)( $\beta$ 1)) is

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involved.  
ABSTRACT WORD COUNT: 61

LANGUAGE (Publication,Procedural,Application): English; English; English  
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	200219	3201
SPEC A	(English)	200219	31788
Total word count - document A			34989
Total word count - document B			0
Total word count - documents A + B			34989

6/3, AB/25 (Item 3 from file: 348)  
DIALOG(R) File 348:EUROPEAN PATENTS  
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01388766

Targeting through integrins  
Zielgerichtete Gentherapie durch Integrine  
Ciblage par les intégrines  
PATENT ASSIGNEE:

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Scholte, Bob Johan, Veenmos 4, 3069 AT Rotterdam, (NL)

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PATENT (CC, No, Kind, Date): EP 1178117 A1 020206 (Basic)

APPLICATION (CC, No, Date): EP 2000202750 000802;

DESIGNATED STATES: DE

EXTENDED DESIGNATED STATES: AL; LT; LV; MK; RO; SI

INTERNATIONAL PATENT CLASS: C12N-015/87; C12N-015/88; C12N-015/86;  
A61K-048/00

ABSTRACT EP 1178117 A1

The present invention relates to a gene delivery vehicle comprising a general nucleic acid binding domain and an integrin binding domain. Typically, the at least two different domains of the gene delivery vehicle are somehow linked. Said gene delivery vehicle may be used to deliver a therapeutic molecule to a cell exposing integrin, thereby at least in part enhancing the efficacy of gene delivery using integrin receptors. In one embodiment, an integrin binding domain of the invention is derived from the C-terminal region of the *Yersinia pseudotuberculosis* "invasin"\*\* or a functional homologue thereof.

ABSTRACT WORD COUNT: 94

NOTE:

Figure number on first page: 1B

LANGUAGE (Publication,Procedural,Application): English; English; English  
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	200206	383
SPEC A	(English)	200206	8702
Total word count - document A			9085
Total word count - document B			0
Total word count - documents A + B			9085

09/830026

6/3,AB/26 (Item 4 from file: 348)  
DIALOG(R) File 348:EUROPEAN PATENTS  
(c) 2003 European Patent Office. All rts. reserv.

01161996  
METHOD FOR THE PRODUCTION OF \*PURIFIED\*\*\* \*INVASIN\*\*\* PROTEIN AND USE  
THEREOF  
METHODE FUR DIE HERSTELLUNG VON GEREINIGTEM INVASINPROTEIN UND SEINE  
ANWENDUNG  
PROCEDE DE PRODUCTION DE PROTEINE \*INVASINE\*\*\* HAUTEMENT \*PURIFIEE\*\*\* ET  
UTILISATION DE CETTE PROTEINE

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PATENT (CC, No, Kind, Date): EP 1131338 A1 010912 (Basic)  
WO 200023462 000427

APPLICATION (CC, No, Date): EP 99970664 991021; WO 99US24931 991021

PRIORITY (CC, No, Date): US 105085 P 981021; US 136754 P 990601

DESIGNATED STATES: AT; BE; CH; CY; DE; DK; ES; FI; FR; GB; GR; IE; IT; LI;  
LU; MC; NL; PT; SE

EXTENDED DESIGNATED STATES: AL; LT; LV; MK; RO; SI

INTERNATIONAL PATENT CLASS: C07K-001/00; A61K-038/00; A61K-039/00;  
A61K-039/02; A61K-039/116; A61K-045/00; A61K-039/085

NOTE:

No A-document published by EPO

LANGUAGE (Publication,Procedural,Application): English; English; English

6/3,AB/27 (Item 5 from file: 348)  
DIALOG(R) File 348:EUROPEAN PATENTS  
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PATENT (CC, No, Kind, Date): EP 872555 A1 981021 (Basic)  
WO 9706264 970220

09/830026

APPLICATION (CC, No, Date): EP 96925994 960805; WO 96JP2196 960805  
PRIORITY (CC, No, Date): JP 95199745 950804  
DESIGNATED STATES: CH; DE; GB; LI  
INTERNATIONAL PATENT CLASS: C12N-015/62; C12N-015/31; C12Q-001/02;  
C12N-001/21; C07K-019/00;

ABSTRACT EP 872555 A1

The present invention relates to a fusion protein specifically recognizing a particular target cell or protein. The fusion protein comprises a protein derived from a cell invasive protein (support protein) to be a support and a protein to be an object (object protein). This invention also relates to a bacterium presenting the fusion protein on its surface, and to a bacterial peptide library as a collection of the bacteria. The present invention permits to add desirable biological activities, such as binding ability to cells or proteins, invading ability into cells, to the fusion protein. Also, the bacteria of the present invention can be easily self-reproducible and is stable against any handling without degradation.

ABSTRACT WORD COUNT: 114

LANGUAGE (Publication, Procedural, Application): English; English; Japanese  
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	9843	2132
SPEC A	(English)	9843	16583
Total word count - document A			18715
Total word count - document B			0
Total word count - documents A + B			18715

6/3, AB/28 (Item 6 from file: 348)  
DIALOG(R) File 348: EUROPEAN PATENTS  
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00823317

ORAL DOSAGE COMPOSITION COMPRISING ZONULA OCCLUDENS TOXIN AND A BIOLOGICALLY ACTIVE INGREDIENT AND USE THEREOF  
ORALE ZUBEREITUNG ENTHALTEND ZONULA OCCLUDENS TOXIN UND EINEN BIOLOGISCH AKTIVEN WIRKSTOFF SOWIE DEREN VERWENDUNG  
COMPOSITION ADMINISTREEE PAR VOIE ORALE COMPRENNANT UNE TOXINE DE ZONULA OCCLUDENS ET UN AGENT AYANT UNE ACTIVITE BIOLOGIQUE ET SON UTILISATION  
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PATENT (CC, No, Kind, Date): EP 828481 A1 980318 (Basic)  
EP 828481 A1 990609  
EP 828481 B1 030402  
WO 96037196 961128

APPLICATION (CC, No, Date): EP 96914626 960516; WO 96US6870 960516  
PRIORITY (CC, No, Date): US 443864 950524; US 598852 960209  
DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FI; FR; GB; GR; IE; IT; LI; LU;  
MC; NL; PT; SE  
INTERNATIONAL PATENT CLASS: A61K-009/20; A61K-047/42; C07K-014/28;

09/830026

C07K-014/705; A61P-003/10

NOTE:

No A-document published by EPO

LANGUAGE (Publication, Procedural, Application): English; English; English

FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS B	(English)	200314	818
CLAIMS B	(German)	200314	679
CLAIMS B	(French)	200314	850
SPEC B	(English)	200314	13292
Total word count - document A			0
Total word count - document B			15639
Total word count - documents A + B			15639

6/3,AB/29 (Item 1 from file: 357)  
DIALOG(R)File 357:Derwent Biotech Res.  
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0197899 DBR Accession No.: 96-08670 PATENT  
Therapeutic delivery system - recombinant invasin production via membrane binding protein fusion protein gene expression in Escherichia coli; application for enhancing systemic bioavailability of therapeutic agents

AUTHOR: Habberfield A D; Jensen-Pippo K  
CORPORATE SOURCE: Thousand Oaks, CA, USA.

PATENT ASSIGNEE: Amgen 1996

PATENT NUMBER: WO 9613250 PATENT DATE: 960509 WPI ACCESSION NO.: 96-251447 (9625)

PRIORITY APPLIC. NO.: WO 94331393 APPLIC. DATE: 941027

NATIONAL APPLIC. NO.: WO 95US13749 APPLIC. DATE: 951020

LANGUAGE: English

ABSTRACT: A therapeutic delivery system for delivery of therapeutic agents (TAs) comprises: i. a TA; and ii. an invasion proficient bacterial protein to transport the composition across the gastrointestinal (GI) membrane barrier by transcytosis, to increase the bioavailability of the TA. The DNA sequence and protein sequence of the bacterial invasin protein are specified. In a preferred system, the transcytosis by the bacterial protein increases the systemic bioavailability of the agent by 5- to 100-fold. The bacterial protein is preferably invasin protein, especially attachment-invasion-locus protein. The TA and bacterial protein are linked by a degradable peptide sequence to form a fusion moiety. The delivery system, utilizing a bacterial invasin protein, allows the efficient delivery, absorption and hence increased bioavailability of orally administered TAs. In an example, nucleic acid encoding the invasin protein fused to membrane binding protein was transfected into Escherichia coli. The expressed protein was extracted from the bacteria and purified with affinity chromatography and cross-linked amylose. (110pp)

Set	Items	Description
S7	104	AU=(PICKING W? OR PICKING, W?)
S8	75	AU=(OAKS, E? OR OAKS E?)
S9	3	S7 AND S8
S10	11	(S7 OR S8) AND S1
S11	9	(S9 OR S10) NOT S5
S12	4	RD (unique items)

- Author(s)

>>>No matching display code(s) found in file(s): 65, 113

09/830026

12/3,AB/1 (Item 1 from file: 144)  
DIALOG(R)File 144:Pascal  
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12731028 PASCAL No.: 96-0439646  
Soluble invasion plasmid antigen C (IpaC) from *Shigella flexneri* elicits epithelial cell responses related to pathogen invasion  
MARQUART M E; \*PICKING W L"\*\*; \*PICKING W D"\*\*  
Department of Biology, Saint Louis University, St. Louis, Missouri  
63103-2010, United States

Journal: Infection and immunity, 1996, 64 (10) 4182-4187

Language: English

*Shigella flexneri* invades colonic epithelial cells by pathogen-induced phagocytosis. The three proposed effectors of *S. flexneri* internalization are invasion plasmid antigens B (IpaB), IpaC, and IpaD, which are encoded on the pathogen's 230-kb virulence plasmid and translocated to the extracellular milieu via the Mxi-Spa translocon. To date, there are no definitive functional data for any purified Ipa protein. Here, we describe the first characterization of highly purified recombinant IpaC, which elicits numerous epithelial cell responses related to events that take place during pathogen invasion. SUP 1 SUP 2 SUP 5 I-labeled IpaC binds cultured Henle 407 intestinal cells with an apparent dissociation constant in the low micromolar range. Moreover, incubation of epithelial cells with IpaC results in general changes in cellular phosphoprotein content, demonstrating this protein's ability to influence cellular protein kinase activities. These results contrast dramatically with those seen for recombinant IpaD, which does not bind to or induce detectable changes in the normal activities of cultured epithelial cells. In addition to influencing host cell activities, preincubation of epithelial cells with purified IpaC enhances uptake of *S. flexneri* by host cells. A similar result is seen when the cells are preincubated with a highly concentrated water extract of virulent *S. flexneri* 2a (strain 2457T). Interestingly, soluble IpaC also appears to promote uptake of the noninvasive *S. flexneri* 2a strain BS103. Purified IpaD failed to enhance the uptake of virulent *S. flexneri* and did not facilitate uptake of BS103. Taken together, the data suggest that IpaC is a potential effector of the host cell biological activities and may be responsible for entry of *S. flexneri* into target cells.

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12/3,AB/2 (Item 1 from file: 440)  
DIALOG(R)File 440:Current Contents Search(R)  
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11342728 References: 57

TITLE: Production of IFN-gamma and IL-10 to *Shigella* \*invasins"\*\* by mononuclear cells from volunteers orally inoculated with a Shiga toxin-deleted *Shigella dysenteriae* type 1 strain  
AUTHOR(S): Samandari T; Kotloff KL; Losonsky GA; \*Picking WD"\*\*; Sansonetti PJ; Levine MM; Sztein MB (REPRINT)  
AUTHOR(S) E-MAIL: msztein@medicine.umaryland.edu  
CORPORATE SOURCE: Univ Maryland, Sch Med, 685 W Baltimore St, Room 480/Baltimore//MD/21201 (REPRINT); Univ Maryland, Sch Med, /Baltimore//MD/21201; Univ Maryland, Sch Med, /Baltimore//MD/21201; St Louis Univ, Dept Biol, /St Louis//MO/63103; INSERM, Unite Pathogenie

09/830026

Microbienne Mol, /Paris//France/  
PUBLICATION TYPE: JOURNAL  
PUBLICATION: JOURNAL OF IMMUNOLOGY, 2000, V164, N4 (FEB 15), P2221-2232  
GENUINE ARTICLE#: 283VK  
PUBLISHER: AMER ASSOC IMMUNOLOGISTS, 9650 ROCKVILLE PIKE, BETHESDA, MD  
20814 USA  
ISSN: 0022-1767  
LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: Volunteers were orally administered invasive, non-Shiga toxin-producing *Shigella dysenteriae* 1 to establish a challenge model to assess vaccine efficacy. In stepwise fashion, four separate groups were given  $3 \times 10(2)$ ,  $7 \times 10(3)$ ,  $5 \times 10(4)$ , or  $7 \times 10(5)$  CFU. Using PBMC, proliferative responses and cytokine production were measured to *S. dysenteriae* whole-cell preparations and to purified recombinant invasion plasmid Ags (Ipa) C and IpaD. Anti-LPS and anti-Ipa Abs and Ab-secreting cells were also evaluated. Preinoculation PBMC produced considerable quantities of IL-10 and IFN-gamma, probably secreted by monocytes and NK cells, respectively, of the innate immune system. Following inoculation, PBMC from 95 and 87% of volunteers exhibited an increased production of IFN-gamma and IL-10, respectively, in response to *Shigella* Ags. These increases included responses to IpaC and IpaD among those volunteers receiving the lowest inoculum. No IL-4 or IL-5 responses were detected. Whereas there were no Ab or Ah-secreting cell responses in volunteers receiving the lowest inoculum, other dose groups had moderate to strong anti-LPS and anti-Ipa responses. These results suggest that in humans, type I responses play an important role in mucosal and systemic immunity to *S. dysenteriae* 1.

12/3, AB/3 (Item 2 from file: 440)  
DIALOG(R) File 440: Current Contents Search(R)  
(c) 2003 Inst for Sci Info. All rts. reserv.

08001629 References: 33  
TITLE: Cloning, expression, and affinity purification of recombinant *Shigella flexneri* invasion plasmid antigens IpaB and IpaC  
AUTHOR(S): \*Picking WL\*\*\*; Mertz JA; Marquart ME; \*Picking WD (REPRINT)\*\*\*  
CORPORATE SOURCE: ST LOUIS UNIV, DEPT BIOL, 3507 LACLEDE AVE/ST  
LOUIS//MO/63103 (REPRINT); ST LOUIS UNIV, DEPT BIOL/ST LOUIS//MO/63103  
PUBLICATION TYPE: JOURNAL  
PUBLICATION: PROTEIN EXPRESSION AND PURIFICATION, 1996, V8, N4 (DEC), P  
401-408  
GENUINE ARTICLE#: VX143  
PUBLISHER: ACADEMIC PRESS INC JNL-COMP SUBSCRIPTIONS, 525 B ST, STE 1900,  
SAN DIEGO, CA 92101-4495  
ISSN: 1046-5928  
LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: *Shigella flexneri* and related enteropathogenic bacteria are important agents of bacillary dysentery, a potentially life-threatening illness for children in underdeveloped regions of the world. Onset of shigellosis stems from *S. flexneri* invasion of colonic epithelial cells, leading to localized cell death and inflammation. Invasion plasmid antigens (Ipa) B, C, and D are three secreted proteins encoded by the large virulence plasmid of *S. flexneri* that have been implicated as essential effectors of this cell invasion process. These proteins are expressed as part of the ipa operon and are among the major targets of the host immune

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response to shigellosis. Biochemical characterization of the Ipa "invasins"\*\* has been complicated by the fact they have not been purified in the quantities needed for detailed in vitro analysis. Here we describe the first cloning, expression, and extensive purification of IpaB and IpaC fusion proteins from Escherichia coli for use in dissecting of the protein biochemistry of S. flexneri pathogenesis. A variety of approaches were used to prepare significant quantities of these proteins in their soluble forms, including the use of different host cell lines, modification of bacterial growth conditions, and the use of alternative plasmid expression vectors. Now that these Ipa proteins are available in a highly pure form, it will be possible to initiate studies on their important biological and immunological properties as well as their recruitment into high-molecular-weight protein complexes. Together with IpaD (purified as part of a previous study), these purified proteins will be useful for: (a) exploring properties of the host immune response to S. flexneri invasion, (b) elucidating the specific biochemical properties that lead to pathogen internalization, (c) analyzing the importance of specific Ipa protein complexes in host cell invasion, and (d) monitoring, or perhaps even augmenting, the efficacy of live oral vaccines in human trials. (C) 1996 Academic Press, Inc.

12/3,AB/4 (Item 3 from file: 440)  
DIALOG(R)File 440:Current Contents Search(R)  
(c) 2003 Inst for Sci Info. All rts. reserv.

07187519 References: 15  
TITLE: ANTIBODY RESPONSE OF MONKEYS TO INVASION PLASMID ANTIGEN D AFTER  
INFECTION WITH SHIGELLA spp  
AUTHOR(S): \*OAKS EV"\*\*; \*PICKING WD"\*\*; \*PICKING WL"\*\*  
CORPORATE SOURCE: WALTER REED ARMY MED CTR,WALTER REED ARMY INST RES,DEPT  
ENTER INFECT/WASHINGTON//DC/20307 (Reprint); ST LOUIS UNIV,DEPT BIOL/ST  
LOUIS//MO/63103  
PUBLICATION: CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, 1996, V3, N2 (MAY), P242-245  
GENUINE ARTICLE#: TY857  
ISSN: 1071-412X  
LANGUAGE: ENGLISH DOCUMENT TYPE: ARTICLE

ABSTRACT: The antigen preparation most often used for determining the levels of antibodies to virulence-associated proteins of Shigella spp. consists of a mixture of proteins (including IpaB, IpaC, IpaD, and VirG\*) extracted from virulent shigellae with water (water extract). To overcome the lack of specificity for individual antigens in the water-extract enzyme-linked immunosorbent assay (ELISA), the ipaD gene from S. flexneri has been cloned, expressed to a high level, acid purified for use in a nem ELISA for the determination of the levels of antibody against IpaD in monkeys and humans challenged with shigellae. The IpaD ELISA for serum immunoglobulins G and A correlated well with the water-extract ELISA in that monkeys infected with S. flexneri or S. sonnei responded with high serum antibody titers in both assays. The IpaD assay required less antigen per well, had much lower background levels, and did not require correction with antigens from an avirulent organism. In conjunction with the water-extract ELISA, it was possible to identify infected animals that did not respond to IpaD but did produce antibodies that reacted in the water-extract ELISA. This indicates that even though IpaB, IpaC, and IpaD are essential for the invasiveness phenotype, the infected host does not always produce antibodies against all components of the invasiveness

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